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To: Marcus Plescia, MD, MPH

Health Director

Mecklenburg County Health Department

From: Megan Davies, MD

State Epidemiologist

North Carolina Department of Health and Human Services

Larry Michael, REHS, MPH

State Environmental Health Director

North Carolina Department of Health and Human Services

Re: Public health investigation of a primary amebic meningoencephalitis case

associated with exposure to Naegleria fowleri at the U.S. National Whitewater Center in

Mecklenburg County, North Carolina

Background

On June 19, 2016, the North Carolina Division of Public Health (NC DPH) was notified by the Centers for Disease Control and Prevention (CDC) of a suspected case of primary amebic meningoencephalitis in an Ohio resident who had water exposures in North Carolina before illness onset. On June 21, 2016, *Naegleria fowleri* was confirmed by polymerase chain reaction (PCR) testing performed by CDC on a sample of the patient's cerebrospinal fluid. An investigation was launched to identify potential sources of exposure and assess the potential for ongoing risks.

Epidemiologic and Environmental Investigation Methods

Detailed information regarding the patient's illness and all potential sources of exposure to *N. fowleri* during the incubation period (2–9 days before illness onset) was obtained from the Ohio Department of Health and from local public health officials in the patient's county of residence. Information was based on interviews with the patient's family and with other persons who were with the patient during the likely incubation period.

Based on exposure information obtained from these sources (summarized below), an on-site visit to the U.S. National Whitewater Center (USNWC) in Charlotte, NC was conducted on June 22, 2016 with staff from CDC, the Mecklenburg County Health Department, and NC DPH. This on-site assessment included a visual inspection of the facilities, discussions with USNWC staff, and measurement of selected physical and chemical water parameters. Samples were collected for *N. fowleri* testing at CDC, including water, filter backwash and surface swab samples from locations within the USNWC and water, sediment and surface swab samples from the adjacent Catawba River.

Public health notifications describing the *N. fowleri* infection and exposure to USNWC were distributed statewide through direct communication with all local health departments and nationally through the CDC's Epidemic Information Exchange (Epi-X). Information was also disseminated to the public through local media releases and through media interviews and availabilities.

Results

Clinical and epidemiologic information: The patient developed initial symptoms of headache and congestion on June 14, 2016, three days after returning from an 8-day youth trip that involved travel to North Carolina and West Virginia. The patient was admitted to the hospital two days after illness onset with suspected meningitis and died on June 19, 2016. On June 21, 2016, N. fowleri was confirmed by PCR testing performed by CDC on a sample of the patient's cerebrospinal fluid.

A detailed investigation by public health officials in Ohio identified no water exposures that would present opportunities for exposure to *N. fowleri* during the incubation period (approximately June 5–12, 2016) with the exception of a whitewater rafting event at USNWC on June 8, 2016. The patient had fallen out of the raft and been submerged during the rafting event.

Facility description: The USNWC is located in Charlotte, NC on 1,100 acres near the Catawba River. Activities offered include whitewater rafting and kayaking, flatwater kayaking, stand-up paddle boarding, rock climbing, zip lines, ropes courses, a canopy tour, and mountain biking. Whitewater activities take place in a recirculating artificial whitewater feature. The facility is not subject to North Carolina public swimming pools laws and rules. The channel structures for the whitewater feature are poured concrete with a geobarrier and water barrier membrane below the concrete to prevent mixing with groundwater. Water for filling and maintaining water levels in the whitewater feature is obtained from onsite wells and Mecklenburg County municipal water. At no place is river water introduced into the system. Storm drains and ground

drainage around the site are graded to minimize runoff from entering the system. However, the channels are open, so water levels do increase during rain storms.

The whitewater feature contains approximately 12 million gallons at any time. Seven pumps are available; one is kept in reserve and six are usually used to lift the water approximately 21' to an upper pond, and water then flows by gravity down one of two channels. An eighth pump separate from those that create the water flow down the channels pulls the water from the lower pond into a filtration unit. The flow of this pump (approximately 8,600 gallons per minute per facility staff) is intended to bring the total volume of the system through filtration once every 24 hours. There are four filters consisting of interlocking plastic disks with a pore size of 200 microns. Monitors automatically backwash the filters when the pressure gradient on each side of the filter exceeds a preset value. After filtration, the water is forced through an ultraviolet (UV) disinfection point. Eighteen UV tubes project through a two-foot section of pipe. From the filter unit, water is discharged to upper pond.

Chlorine is added as a disinfectant only when coliform counts are trending upward or algae growth has visibly increased. Chlorine is added as a liquid to water in the upper pond.

Areas where sediment collects are mucked and vacuumed out from the upper pond and upper runs when the pumps are off on an as needed basis. Water settling in the lower pond prevents removal of sediment from that location during the operational season.

The system is drained and cleaned annually. Scrubbing and pressure washing are used to clean the concrete and rock surfaces. The system is left dry for approximately two months each year.

The facility has a contract with a commercial laboratory to perform weekly testing. Fecal coliform counts, turbidity, pH, temperature and a number of heavy metals are measured.

Site visit and laboratory findings: Findings from the site visit to USNWC on June 22, 2016 are summarized in the appendix. Briefly, measurements obtained from the lower pond at USNWC included a low level of free chlorine (0.05 mg/L), an elevated level of turbidity (6.7 nephelometric turbidity units [NTU]), and a water temperature of 86°F. Facility staff reported that the frequency with which the automated filter backwash was triggered varied, but could be as frequently as every six minutes due to the amount of sediment in the system.

All 11 samples collected from USNWC were PCR-positive for *N. fowleri*. One water sample from the top pond was strongly PCR-positive with a cycle threshold (Ct) value \leq 30. This indicates a higher concentration of *N. fowleri* than has been detected previously from open water sources, for which Ct values have been \geq 36. Ten of the 11 USNWC samples were also PCR-positive following a culture assay, indicating that the *N. fowleri* organisms in these samples were viable. No culture data were available from the 11th sample.

All four samples collected from or near the Flatwater Dock in the Catawba River (two water samples and two swabs from the dock) were PCR-negative for *N. fowleri*. The single sediment sample from the river was PCR-positive for *N. fowleri* following the culture assay.

Control Measures

After consultation with public health officials, the US National Whitewater Center decided to voluntarily close their whitewater feature on Friday, June 24, 2016.

Discussion

A thorough review of clinical and epidemiologic information indicates that the fatal infection occurred following exposure to *N. fowleri* during a rafting event at USNWC. Findings from the on-site assessment at USNWC and laboratory testing indicate that:

- *N. fowleri* were present throughout the whitewater feature;
- Conditions were conducive to growth and amplification of N. fowleri; and
- Water treatment modalities in use were not effective against this pathogen.

It is likely that effectiveness of the UV disinfection system in use was limited by the flow dynamics and turbidity of the water, and that effectiveness of chlorine disinfection was limited by the high organic load in the water. Moreover, the filtration system in use did not appear to be sufficient to remove the amount of sediment in the system and had a pore size that was not intended for removal of microorganisms.

The combination of high levels of *N. fowleri* and the likelihood of submersion and exposure to high-velocity water result in a risk of infection that is likely higher than the risk of infection from exposure to *N. fowleri* in the natural environment.

Recommendations

The North Carolina Division of Public Health recommends that the USNWC continue to keep the whitewater feature closed until an acceptable remediation strategy and ongoing water safety plans have been devised for reducing the risk of *N. fowleri* infection. These plans should be developed in consultation with experts in large-scale recreational water safety, and should be reviewed by public health authorities prior to implementation.

Ongoing testing of water quality parameters should be performed after remediation and implementation of agreed-upon plans to ensure that conditions favorable to the growth and amplification of *N. fowleri* are not permitted to re-occur.

APPENDIX: Summary of Environmental Testing by CDC at the U.S. National Whitewater Center and the Catawba River near Flatwater Dock

Summary of Environmental Testing by CDC at the US National Whitewater Center (WWC) and the Catawba River near Flatwater Dock

Prepared by: Jennifer Murphy, PhD; Amy Kahler, MS; Jothikumar, Narayanan, PhD; Vincent Hill, PhD Date: 06/30/16

Methods

Select physical and chemical parameters were measured in water at the WWC's lower pond and water surrounding the Flatwater Dock in the Catawba River on 6/22/16.

On 6/22/16, water, filter backwash, and surface swab samples were collected within the WWC and water, sediment, and surface swab samples were collected from or near the Flatwater Dock in the Catawba River (see Figure). Samples were transported and stored in the laboratory at ambient temperature. Sample processing began on the morning of 6/23/16. Water, filter backwash, and sediment samples were processed using a concentration procedure; surface swab samples were processed using an elution and concentration procedure. Concentrates were divided; one aliquot was submitted to a *Naegleria fowleri* (*N. fowleri*) real-time polymerase chain reaction (PCR) assay and a second aliquot was submitted to a culture assay. Aliquots of top pond samples and the Catawba River sediment sample were also submitted directly (i.e., without prior concentration) to the culture assay. If trophozoite or cyst migration was visible on a culture plate within 7 days of incubation at 44°C, material from the plate was harvested and submitted to the real-time PCR assay for confirmation of viable *N. fowleri*.

Results

Table 1: Physical and chemical test results for WWC lower pond and Catawba River

	Free chlorine	Total chlorine	Turbidity	Water Temperature
	residual (mg/L)	residual (mg/L)	(NTU)	(°C/°F)
WWC lower pond	0.05	0.15	6.7	29.6/85.3
Catawba River	N/A	N/A	4.1	28.3/83.0

NTU: Nephelometric Turbidity Units

N/A: not applicable; free and total chlorine residuals are not typically measured in natural waters

Table 2: N. fowleri real-time PCR results for samples collected within WWC and from or near the Flatwater Dock in the Catawba River

Sample ID	Sample Type	Direct Real-time PCR Results*	Culture Real-time PCR Results*			
WWC Samples						
3323	Pod 1 backwash sample (0.75 L)	Positive	Positive [‡]			
3324	Pod 2 backwash sample (0.75 L)	Positive	Positive [‡]			
3325	Pod 3 backwash sample (0.75 L)	Positive	Positive [‡]			
3326	Pod 4 backwash sample (0.75 L)	Positive	Positive [‡]			
3328	Bottom pond small-volume water sample (0.75 L)	Positive	Positive [‡]			
3329	Bottom pond large-volume water sample (50 L)	Positive	Positive [‡]			
3332	Top pond water sample (~0.7 L)	Positive [‡]	Positive [‡]			
3338	Top pond water sample (~0.5 L)	Positive ⁵	ND			
3335	"Wilderness" surface swab sample (4" x 4")	Positive	Positive [‡]			
3336	"Wilderness" plant sample	Positive	Positive [‡]			
3337	Boat loading ramp surface swab sample (4" x 4")	Positive	Positive [‡]			
Flatwater Dock at Catawba River (CR) Samples						
3327	CR small-volume water sample	Negative	Negative			
3330	CR large-volume water sample	Negative	Negative			
3331	CR sediment sample	Negative	Positive [‡]			
3333	Flatwater Dock sub-surface swab sample (4" x 4")	Negative	Negative			
3334	Flatwater Dock above surface swab sample (4" x 4")	Negative	Negative			

^{*}A sample was considered positive for N. fowleri when Ct < 40

Direct real-time PCR only performed on non-concentrated sample

ND: no data are available for this sample

Please note: Real-time PCR is used to determine the amount of a target nucleic acid (e.g., *N. fowleri* DNA) that is present in a sample. The "Ct" (threshold cycle) value is a relative measure of the concentration of the target nucleic acid in the PCR reaction. A Ct value of <40 indicates the presence of the target nucleic acid in the sample (i.e., a positive result). The lower the Ct value, the greater the amount of target nucleic acid in the sample. A Ct value ≤30 is considered a strong positive result (i.e., abundant target nucleic acid was present in the sample). Ct values for pure cultures of microbes are generally in the low- to mid-20s. Ct values ≤30 are generally not detected in environmental samples when they are directly assayed by real-time PCR.

Conclusions

Free and total chlorine residuals in the WWC lower pond were very low—with the free chlorine
results just above the detection limit for the method (0.02 ± 0.05 mg/L). Considering the
relatively high turbidity of the water (relatively high for a system in which chlorination is being
performed), there may have been compounds in the water (e.g., manganese, organic
chloramines) that are potential interferences for the free and total chlorine testing methods and

[‡]Ct value ≤30

the manufacturer (Hach Company) notes potential analytical issues presented by elevated turbidity.

- The testing results indicate that water did not contain sufficient free chlorine residual for disinfection of water (e.g., free chlorine residual >1 mg/L for recreational water and >0.2 mg/L for drinking water)
- Water temperature in both the WWC and in the Catawba River at the Flatwater Dock were >80°F; N. fowleri is most commonly found in freshwaters with elevated temperatures (i.e., 80-115°F).
- The turbidity level in the WWC lower pond was too high for the water to maintain sufficient chlorine residual; turbidity levels in properly-chlorinated recreational water venues should not exceed 0.5 NTU.
- WWC N. fowleri test results:
 - All 11 samples collected from the WWC were PCR-positive for N. fowleri.
 - One top pond water sample was strongly PCR-positive for N. fowleri (Ct value ≤30).
 - The test results indicate a higher concentration of Naegleria than we have seen
 in samples taken from the natural environment.
 - 10 of the 11 WWC samples were PCR-positive for N. fowleri following the culture assay;
 no culture data are available for the eleventh sample.
 - These results indicate that N. fowleri in these 10 PCR-positive samples was viable.
- Flatwater Dock/Catawba River N. fowleri test results:
 - All five samples collected from or near the Flatwater Dock were PCR-negative for N. fowleri.
 - However, the sediment sample was PCR-positive for N. fowleri following the culture assay.
 - The direct PCR was negative, possibly due to the presence of PCR inhibitors in the sample or the concentration of N. fowleri being below the method's detection limit.
 - Detecting N. fowleri in natural water body sediments is not unusual in southerntier states during warm weather months.

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Figure: Sample collection locations at the US National Whitewater Center and the Flatwater Dock in the Catawba River